Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1644PNH

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2		"Ask CAS" for self-help around the clock
NEWS 3	JAN 27	Source of Registration (SR) information in REGISTRY updated and searchable
NEWS 4	JAN 27	A new search aid, the Company Name Thesaurus, available in CA/CAplus
NEWS 5	FEB 05	German (DE) application and patent publication number format changes
NEWS 6	MAR 03	MEDLINE and LMEDLINE reloaded
NEWS 7	MAR 03	MEDLINE file segment of TOXCENTER reloaded
NEWS 8	MAR 03	FRANCEPAT now available on STN
NEWS 9	MAR 29	Pharmaceutical Substances (PS) now available on STN
NEWS 10	MAR 29	WPIFV now available on STN
NEWS 11	MAR 29	New monthly current-awareness alert (SDI) frequency in RAPRA
NEWS 12	APR 26	PROMT: New display field available
NEWS 13	APR 26	IFIPAT/IFIUDB/IFICDB: New super search and display field available
NEWS 14	APR 26	LITALERT now available on STN
NEWS 15	APR 27	NLDB: New search and display fields available
NEWS 16	May 10	PROUSDDR now available on STN
NEWS 17	May 19	PROUSDDR: One FREE connect hour, per account, in both May and June 2004
NEWS 18	May 12	EXTEND option available in structure searching
NEWS 19	May 12	Polymer links for the POLYLINK command completed in REGISTRY
NEWS 20	May 17	FRFULL now available on STN
NEWS 21	May 27	STN User Update to be held June 7 and June 8 at the SLA 2004 Conference
NEWS 22	May 27	New UPM (Update Code Maximum) field for more efficient patent SDIs in CAplus
NEWS 23	May 27	CAplus super roles and document types searchable in REGISTRY
NEWS 24	May 27	Explore APOLLIT with free connect time in June 2004

NEWS EXPRESS	MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

```
  * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 12:49:58 ON 16 JUN 2004

=> file medline embase biosis scisearch caplus
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.21       0.21

FILE 'MEDLINE' ENTERED AT 12:50:09 ON 16 JUN 2004

FILE 'EMBASE' ENTERED AT 12:50:09 ON 16 JUN 2004
COPYRIGHT (C) 2004 Elsevier Inc. All rights reserved.

FILE 'BIOSIS' ENTERED AT 12:50:09 ON 16 JUN 2004
COPYRIGHT (C) 2004 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'SCISEARCH' ENTERED AT 12:50:09 ON 16 JUN 2004
COPYRIGHT 2004 THOMSON ISI

FILE 'CAPLUS' ENTERED AT 12:50:09 ON 16 JUN 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

=> s monoclonal antibod?
L1      658278 MONOCLONAL ANTIBOD?

=> s l1 and conformation epitpe
L2           0 L1 AND CONFORMATION EPITPE

=> s l1 and human TSH receptor
L3         135 L1 AND HUMAN TSH RECEPTOR

=> s l3 and conformation epitope
L4           0 L3 AND CONFORMATION EPITOPE

=> s l3 and conformational
L5          12 L3 AND CONFORMATIONAL

=> dup remove l5
PROCESSING COMPLETED FOR L5
L6           6 DUP REMOVE L5 (6 DUPLICATES REMOVED)

=> d l6 1-6 cbib abs

L6   ANSWER 1 OF 6  SCISEARCH  COPYRIGHT 2004 THOMSON ISI on STN
2003:332358   The Genuine Article (R) Number: 668BE. The cysteine-rich amino
     terminus of the thyrotropin receptor is the immunodominant linear antibody
     epitope in mice immunized using naked deoxyribonucleic acid or adenovirus
     vectors.  Schwarz-Lauer L; Pichurin P N; Chen C R; Nagayama Y; Paras C;
     Morris J C; Rapoport B; McLachlan S M (Reprint). Cedars Sinai Res Inst,
     Autoimmune Dis Unit, Los Angeles, CA 90048 USA (Reprint); Univ Calif Los
     Angeles, Sch Med, Los Angeles, CA 90048 USA; Nagasaki Univ, Dept Pharmacol
     1, Sch Med, Nagasaki 8528523, Japan; Mayo Clin, Div Endocrinol & Metab,
     Dept Med, Rochester, MN 55902 USA. ENDOCRINOLOGY (MAY 2003) Vol. 144, No.
     5, pp. 1718-1725. Publisher: ENDOCRINE SOC. 4350 EAST WEST HIGHWAY SUITE
     500, BETHESDA, MD 20814-4110 USA. ISSN: 0013-7227. Pub. country: USA;
     Japan. Language: English.
     *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB      Experimental Graves' disease is more effectively produced by
     immunization approaches involving in vivo TSH receptor (TSHR) expression
     than by conventional immunization with TSHR protein and adjuvant. Unlike
     conformational epitopes that are extremely difficult to define,
```

linear epitopes can be readily assessed using synthetic peptides. TSHR linear epitopes are well characterized in conventionally immunized animals, but there is no information for animals vaccinated with TSHR DNA in plasmid or adenovirus vectors. We used synthetic peptides to characterize linear epitopes in mice immunized by in vivo expression of TSHR DNA. TSHR adenovirus-injected mice had higher antibody levels than TSHR DNA-vaccinated mice. However, the dominant peptide recognized in both groups was the TSHR cysteine-rich N terminus (residues 22-41). Sera from TSHR adenovirus-immunized (but not TSHRDNA-vaccinated) mice interacted to a lesser extent with peptides encompassing residues 352-401, which include the region deleted following TSHR cleavage as well as the ectodomain juxta-membrane region. Although antibodies characterized using synthetic peptides are probably TSH blockers or nonfunctional, stimulating antibodies may recognize linear components in a **conformational** epitope. The cysteine-rich TSHR N terminus is functionally important in the action of stimulating TSHR autoantibodies in humans. The immunodominance of the same region in immunized mice suggests that this region may also be immunodominant in humans.

L6 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
1999:133698 Document No.: PREV199900133698. A mouse **monoclonal antibody** to a thyrotropin receptor ectodomain variant provides insight into the exquisite antigenic **conformational** requirement, epitopes and in vivo concentration of human autoantibodies. Chazenbalk, Gregorio D.; Wang, Yan; Guo, Jin; Hutchison, J. Scott; Segal, Dean; Jaume, Juan Carlos; McLachlan, Sandra M.; Rapoport, Basil [Reprint author]. Cedars-Sinai Medical Center, 8700 Beverly Blvd., Suite B-131, Los Angeles, CA 90048, USA. Journal of Clinical Endocrinology and Metabolism, (Feb., 1999) Vol. 84, No. 2, pp. 702-710. print.
CODEN: JCEMAZ. ISSN: 0021-972X. Language: English.
AB We used the secreted TSH receptor (TSHR) ectodomain variant TSHR-289 (truncated at amino acid residue 289 with a 6-histidine tail) to investigate properties of TSHR autoantibodies in Graves' disease. Sequential concanavalin A and Ni-chelate chromatography extracted milligram quantities of TSHR-289 (apprx20-40% purity) from the culture medium. Nanogram quantities of this material neutralized the TSH binding inhibitory activity in all 15 Graves' sera studied. We generated a mouse **monoclonal antibody** (mAb), 3BD10, to partially purified TSHR-289. Screening of a TSHR complementary DNA fragment expression library localized the 3BD10 epitope to 27 amino acids at the N-terminus of the TSHR, a cysteine-rich segment predicted to be highly **conformational.** 3BD10 preferentially recognized native, as opposed to reduced and denatured, TSHR-289, but did not interact with the TSH holoreceptor on the cell surface. Moreover, mAb 3BD10 could extract from culture medium TSHR-289 nonreactive with autoantibodies, but not the lesser amount (apprx25%) of TSHR-289 molecules capable of neutralizing autoantibodies. Although the active form of TSHR-289 in culture medium was stable at ambient temperature, stability was reduced at 37degree C, explaining the mixture of active and inactive molecules in medium harvested from cell cultures. In conclusion, studies involving a TSHR ectodomain variant indicate the exquisite **conformational** requirements of TSHR autoantibodies. Even under "native" conditions, only a minority of molecules in highly potent TSHR-289 preparations neutralize patients' autoantibodies. Therefore, Graves' disease is likely to be caused by even lower concentrations of autoantibodies than previously thought. Finally, reciprocally exclusive binding to TSHR-289 by human autoantibodies and a mouse mAb with a defined epitope suggests that the extreme N-terminus of the TSHR is important for autoantibody recognition.

L6 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN
1998:77320 Document No. 128:166099 Genetic immunization against the human thyrotropin receptor causes thyroiditis and allows production of **monoclonal antibodies** recognizing the native receptor.
Costagliola, S.; Rodien, P.; Many, M.-C.; Ludgate, M.; Vassart, G.

(IRIBHN, ULB, Louvain Medical School, Brussels, Belg.). Journal of Immunology, 160(3), 1458-1465 (English) 1998. CODEN: JOIMA3. ISSN: 0022-1767. Publisher: American Association of Immunologists.

AB The generation of Abs recognizing the native structure of the **human TSH receptor** (hTSHR) has been difficult because there is currently no method allowing the purification of correctly folded Ag in the amts. required by classical immunization protocols. The majority of Abs made against the hTSHR react preferentially with denatured mols. We report that a humoral response against the native hTSHR, compatible with mAb production, is elicited in mice by immunization with a DNA construct encoding the receptor. BALB/c mice were inoculated in the anterior tibialis muscle with 100 pg of plasmid DNA harboring the hTSHR cDNA. Eleven weeks after the first injection, 10 mice of 14 showed by FACS anal. a strong IgG response against the hTSHR expressed at the surface of Chinese hamster ovary cells. A clear TSH-binding inhibiting Ig and TSH-blocking Ab activity (competition with TSH binding and TSH activity, resp.) was demonstrated in the majority of sera tested. One serum exhibited a clear stimulating activity. Despite the maintenance of normal circulating free T4 levels in all mice, these bioactivities persisted until 18 wk, in which mice were sacrificed, their thyroids were examined histol., and spleens from two animals were used for mAb production All mice displayed a severe lymphocytic infiltration of their thyroids, composed mostly of activated B cells. Three mAbs were produced against **conformational** epitopes of the hTSHR. We conclude that genetic immunization is an efficient method of generating Abs recognizing the native structure of the hTSHR and a new way of inducing thyroiditis in mice murine.

L6 ANSWER 4 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

1998:825043 The Genuine Article (R) Number: 130VE. Production of bioactive amino-terminal domain of the thyrotropin receptor via insertion in the plasma membrane by a glycosylphosphatidylinositol anchor. Costagliola S; Khoo D; Vassart G (Reprint). FREE UNIV BRUSSELS, FAC MED, IRIBHN, 808 ROUTE LENNIK, B-1070 BRUSSELS, BELGIUM (Reprint); FREE UNIV BRUSSELS, FAC MED, IRIBHN, B-1070 BRUSSELS, BELGIUM; EUROSCREEN SA, B-1070 BRUSSELS, BELGIUM; SINGAPORE GEN HOSP, DEPT ENDOCRINOL, SINGAPORE 0316, SINGAPORE. FEBS LETTERS (9 OCT 1998) Vol. 436, No. 3, pp. 427-433. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0014-5793. Pub. country: BELGIUM; SINGAPORE. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB A chimeric cDNA construct encoding the extracellular amino-terminal domain (ECD) of the thyrotropin receptor fused to the signal for addition of glycosylphosphatidylinositol from the Thy-1 gene directs efficient expression of the ECD at the plasma membrane of transfected CHO cells. A cell lime (GT14) expressing over 10(6) receptors/cell was isolated, which allows direct detection, by flow cytometry, of autoantibodies from the majority of patients with Graves' disease or autoimmune idiopathic myxedema, Treatment of GT14 cells with a glycosylphosphatidylinositol-specific phospholipase C (PI-PLC) releases a soluble 80 kDa molecule which neutralizes the autoantibodies from Graves patients. Whereas it does not bind TSH when released from the cells by PI-PLC in free form, the soluble ECD displays clear TSH binding activity when it is released as a complex with a **monoclonal antibody** recognizing a **conformational** epitope of the ECD, Our results allow production of bioactive ECD of the thyrotropin receptor in high yield, with possible applications in structural analyses. (C) 1998 Federation of European Biochemical Societies.

L6 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 2

95309169. PubMed ID: 7540542. Generation and characterization of **monoclonal antibodies** to the human thyrotropin (TSH) receptor: antibodies can bind to discrete **conformational** or linear epitopes and block TSH binding. Seetharamaiah G S; Wagle N M; Morris J C; Prabhakar B S. (Department of Microbiology and Immunology,

University of Texas Medical Branch, Galveston 77555-1019, USA.)
Endocrinology, (1995 Jul) 136 (7) 2817-24. Journal code: 0375040. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB Splenocytes from female BALB/c mice immunized with a recombinant extracellular domain of the **human TSH receptor** (ETSHR) were used to generate a panel of 23 hybridomas that produce TSHR-specific **monoclonal antibodies** (mAbs). All mAbs were of the immunoglobulin G (IgG) isotype and belonged to different subclasses, including IgG1, IgG2a, and IgG2b. The antibodies bound to the ETSHR with relatively high affinity, and several of them blocked the binding of [125I]TSH to the TSHR, with some showing better blocking than others. Competitive binding studies with a subgroup of 4 biotinylated mAbs showed at least 3 different binding specificities. To determine the TSHR epitopes to which these mAbs were binding, we tested them against 37 overlapping synthetic peptides that span the entire ETSHR. mAb 47, which did not block TSH binding, bound to an epitope represented by amino acid residues 22-30. mAb 28, which had a TSH binding inhibitory index of 20%, bound to an epitope represented by amino acids 32-41. However, mAbs 37 and 49, with TSH binding inhibitory index values of 39% and 43%, respectively, showed no significant reactivity with any of the peptides, suggesting that they react with a **conformational** epitope. Together, these studies showed that mAbs with discrete binding specificities can interact with either linear or **conformational** epitopes and block TSH binding. The availability of these mAbs should facilitate identification of fine structures of the TSHR that are relevant for its function as well as pathogenesis of a number of thyroid disorders mediated by antibodies to TSHR.

L6 ANSWER 6 OF 6 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 3
94:734481 The Genuine Article (R) Number: PR032. **MONOCLONAL-ANTIBODIES** THAT RECOGNIZE THE NATIVE HUMAN THYROTROPIN RECEPTOR.
JOHNSTONE A P (Reprint); CRIDLAND J C; DACOSTA C R; HARFST E; SHEPHERD P S . ST GEORGE HOSP, SCH MED, DEPT CELLULAR & MOLEC SCI, DIV IMMUNOL, CRANMER TERRACE, LONDON SW17 0RE, ENGLAND (Reprint); UNITED MED & DENT SCH, GUYS HOSP, SCH MED, DEPT IMMUNOL, LONDON SE1 9RT, ENGLAND. MOLECULAR AND CELLULAR ENDOCRINOLOGY (NOV 1994) Vol. 105, No. 2, pp. R1-R9. ISSN: 0303-7207. Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Monoclonal antibodies** have been produced that recognize the native human thyrotropin receptor by using a sensitive screening protocol based on flow cytofluorimetry combined with recombinant eukaryotic cells expressing high levels of the full-length functional receptor. The more standard screening method of ELISA preferentially selected antibodies that only reacted with the denatured receptor. Mice were immunized with recombinant receptor produced in either eukaryotic or prokaryotic systems; after screening and cloning, three stable hybridoma lines were established. An IgM antibody (7B5) produced in response to the eukaryotic material recognized only the native receptor (by flow cytofluorimetry) and did not react with denatured material on ELISA or immunoblotting, suggesting that its epitope is **conformational.** In contrast, two IgG1 antibodies (2C11 and 3B12) produced in response to the prokaryotic material recognized both native and denatured receptor (by flow cytofluorimetry, immunoprecipitation and immunoblotting). The use of different recombinant constructs in the immunoblotting procedure allowed the epitopes for both of the IgG1 antibodies to be assigned to the region 125-369. None of the antibodies stimulated production of cAMP by recombinant cells expressing the full-length functional receptor, but one of the IgG1 antibodies (2C11) did inhibit binding of radiolabelled thyrotropin to these same cells. These antibodies, and others that can now be produced with this screening protocol, will help define the relationship between structure and function of this important receptor.

=> s assay
L7 1935691 ASSAY

=> s l7 and TSH receptor autoantibodies
L8 115 L7 AND TSH RECEPTOR AUTOANTIBODIES

=> s l8 and monoclonal
L9 28 L8 AND MONOCLONAL

=> s l9 and conformational
L10 0 L9 AND CONFORMATIONAL

=> dup remove l9
PROCESSING COMPLETED FOR L9
L11 14 DUP REMOVE L9 (14 DUPLICATES REMOVED)

=> d l11 1-14 cbib abs

L11 ANSWER 1 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2003:196989 Document No.: PREV200300196989. Receptor binding **assay** for detecting TSH-receptor auto-antibodies. Bergmann, Andreas [Inventor, Reprint Author]; Struck, Joachim [Inventor]. Berlin, Germany. ASSIGNEE: B.R.A.H.M.S. Aktiengesellschaft, Hennigsdorf, Germany. Patent Info.: US 6537760 March 25, 2003. Official Gazette of the United States Patent and Trademark Office Patents, (Mar 25 2003) Vol. 1268, No. 4. http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133 (ISSN print). Language: English.

AB In a competitive receptor binding **assay** for detecting TSH-receptor auto-antibodies in a biological sample, the sample is reacted in a reaction mixture which contains (i) a TSH-receptor or TSH-receptor preparation; (ii) a primary competitor, for example labelled TSH; and (iii) an agent for separating a complex composed of the TSH-receptor and the elements bound thereto of the reaction mixture from the liquid phase. According to the invention, the reaction is carried out in the presence of at least one **monoclonal** or polyclonal antibody specific against a partial peptide sequence of the TSH eceptor. This specific antibody is used to immobilize a complex of TSH-receptor and primary competitor and/or as secondary competitor for another part of the TSH-receptor auto-antibodies expected in a sample. The primary or secondary competitors are or can be selectively labelled.

L11 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
2001:869653 Document No. 136:368016 Thyroid autoantibodies. Smith, Bernard Rees (RSR Ltd, Cardiff, CF23 8HE, UK). Scandinavian Journal of Clinical and Laboratory Investigation, Supplement, 61(235), 45-52 (English) 2001. CODEN: SCLSAH. ISSN: 0085-591X. Publisher: Taylor & Francis.

AB The characteristics of thyroid autoantibodies are reviewed and new **assays** for the autoantibodies described, in particular point of care (POC) tests for thyroid peroxidase (TPO) autoantibodies and for thyroglobulin (Tg) autoantibodies. These POC tests depend on the ability of the autoantibodies to inhibit gold labeled human **monoclonal** antibodies binding to TPO or to Tg. The POC tests show similar sensitivity and specificity to conventional ELISA for the autoantibodies. A new ELISA to measure autoantibodies to the TSH receptor (TRAb) is described, is based on TSH receptor coated onto plate wells by way of a **monoclonal** antibody. Comparison of porcine and human TSH receptors indicates that there is no advantage in using human TSHR in **assay** systems based on competition between TRAb and bovine or porcine TSH for immobilized TSHR. In terms of the origins of Graves' disease, it is speculated that this most common overt autoimmune disease in man might have occurred first when Homo sapiens sapiens migrated rapidly out of Africa about 100,000 yr ago.

L11 ANSWER 3 OF 14 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:201928 Document No.: PREV200100201928. **TSH receptor autoantibodies.** Smith, Bernard Rees [Reprint author]. RSR Ltd., Cardiff, UK. Endocrine Journal, (August, 2000) Vol. 47, No. Suppl. August,

pp. 99. print.
Meeting Info.: 12th International Thyroid Congress. Kyoto,, Japan. October 22-27, 2000. British Society of Gastroenterology.
ISSN: 0918-8959. Language: English.

L11 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
1999:796053 Document No. 132:34772 **Assays** for **TSH receptor autoantibodies.** Sanders, Jane; Smith, Bernard Rees; Furmaniak, Jadwiga (Rsr Ltd., UK). PCT Int. Appl. WO 9964865 A1 19991216, 25 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1774 19990604. PRIORITY: GB 1998-12146 19980606; GB 1999-9661 19990428.
AB A method of monitoring autoantibodies to TSH (TSH) receptor in a sample of body fluid, comprising the steps of: (a) incubating TSH receptor with a sample of body fluid; (b) reacting the incubated sample of body fluid with at least one binding agent which is capable of binding to the TSH receptor in competitive reaction with **TSH receptor autoantibodies** (TRAb), or in a case where TSH receptor is complexed to labeled antibody, reacting the sample of body fluid with at least one binding agent which can bind to TRAb in such way as not substantially to interfere with binding of the TRAb to the TSH receptor; and (c) detecting bound TRAb in the reacted incubated sample of body fluid. Thus, mol. cloning of TSH receptor cDNA was performed, recombinant porcine TSHR protein was expressed and used for preparation of **monoclonal** anti-TSHR antibody (4E31, IgG), immobilized 4E31 and 123I-labeled TSH-TSH receptor complex were prepared for detecting autoantibody in sera of patients with Graves' disease.

L11 ANSWER 5 OF 14 MEDLINE on STN DUPLICATE 1
1999450867. PubMed ID: 10523032. The interaction of **TSH receptor autoantibodies** with 125I-labelled TSH receptor.
Sanders J; Oda Y; Roberts S; Kiddie A; Richards T; Bolton J; McGrath V; Walters S; Jaskolski D; Furmaniak J; Smith B R. (FIRS Laboratories, RSR Ltd., Llanishen, Cardiff, Wales, United Kingdom.) Journal of clinical endocrinology and metabolism, (1999 Oct) 84 (10) 3797-802. Journal code: 0375362. ISSN: 0021-972X. Pub. country: United States. Language: English.
AB Detergent-solubilized porcine TSH receptor (TSHR) has been labeled with 125I using a **monoclonal** antibody to the C-terminal domain of the receptor. The ability of sera containing TSHR autoantibody to immunoprecipitate the labeled receptor was then investigated. Sera negative for TSHR autoantibody (as judged by **assays** based on inhibition of labeled TSH binding to detergent-solubilized porcine TSHR) immunoprecipitated about 4% of the labeled receptor, whereas sera with high levels of receptor autoantibody immunoprecipitated more than 25% of the labeled receptor. The ability to immunoprecipitate labeled TSHR correlated well with ability of the sera to inhibit labeled TSH binding to the receptor ($r = 0.92$; $n = 63$), and this is consistent with TSHR autoantibodies in these samples being directed principally to a region of the receptor closely related to the TSH binding site. Preincubation of labeled TSHR with unlabeled TSH before reaction with test sera inhibited the immunoprecipitation reaction, providing further evidence for a close relationship between the TSHR autoantibody binding site(s) and the TSH binding site. This was the case whether the sera had TSH agonist (i.e., thyroid stimulating) or TSH antagonist (i.e., blocking) activities, thus, providing no clear evidence for different regions of the TSHR being involved in forming the binding site(s) for TSHR autoantibodies with stimulating and with blocking activities. The ability of TSHR autoantibodies to stimulate cyclic AMP production in isolated porcine thyroid cells was compared with their ability to immunoprecipitate labeled porcine TSHR. A significant correlation was observed ($r = 0.58$; $n = 50$; $P < 0.001$) and the correlation was improved when stimulation of cyclic AMP production was compared with inhibition of labeled TSH binding to porcine TSHR ($r = 0.76$). Overall, our results indicate that TSHR autoantibodies bind principally to a region on the TSHR closely related to the TSH

binding site, and this seems to be the case whether the autoantibodies act as TSH agonists or antagonists.

L11 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
1998:324967 Document No. 129:3853 Receptor binding **assay**, appropriate recombinant fusion receptor for this **assay**, vector for its production and reagent kit for implementing the receptor binding **assay**. Loos, Ulrich; Minich, Waldemar B. (B.R.A.H.M.S Diagnostica G.m.d.H., Germany; Loos, Ulrich; Minich, Waldemar B.). PCT Int. Appl. WO 9820343 A2 19980514, 43 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German). CODEN: PIXXD2. APPLICATION: WO 1997-EP6121 19971105. PRIORITY: DE 1996-19645729 19961106; DE 1997-19728991 19970707.
AB This invention discusses a receptor binding **assay** to determine **TSH receptor autoantibodies** in a biol. sample, in which a preparation of a recombinant TSH fusion receptor as TSH receptor preparation is used, which is extended by one peptide radical as compared to a functional TSH receptor, wherein said peptide radical (i) has a label or can be selectively labeled and/or (ii) can be immobilized or is immovable by binding with a selective binding partner, for instance a **monoclonal** antibody, a metal-chelate resin or a biotin-binding protein without significantly impairing the required functionality of the TSH receptor for the receptor binding **assay**. Further, a recombinant TSH fusion receptor is described which has the amino acid sequence of a functional human TSH receptor, which is extended by one peptide radical with the properties cited supra, specially one which has been produced by using a vaccinia virus/HeLa cells expression system.

L11 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2004 ACS on STN
1998:392719 Document No. 129:53347 Receptor binding **assay** for detection of TSH-receptor antibodies as well as reagents for its execution. Bergmann, Andreas; Struck, Joachim (B.R.A.H.M.S Diagnostica G.m.b.H., Germany). Ger. Offen. DE 19651093 A1 19980610, 12 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1996-19651093 19961209.
AB The invention concerns a competitive immunoassay for the improved diagnosis of Morbus Basedow by detecting **TSH-receptor autoantibodies** from human serum. The method includes parts of the known TRAK **Assay**. The analyte TSH-receptor antibodies are competing for the TSH-receptor with labeled TSH primary competitor and with a secondary competitor, that consists of an antibody against a peptide fraction of the TSH-receptor. The peptide fraction is typical for Morbus Basedow and the usage of the **monoclonal** antibody against it improves the sensitivity of the **assay**. The TSH-receptor is of human, animal or recombinant origin, the competing **assay** is carried out on a solid surface, that are particles, small tubes, microtiter plates from glass or plastic. Thus **monoclonal** antibody was raised against the peptide comprising the amino acid sequence 20-29 of the human TSH-receptor, the antibody was immobilized onto Carbolink Gel and filled into a column. Test solution, porcine TSH-receptor, radioactive iodine labeled TSH were mixed, incubated and loaded onto the column containing the secondary competitor bound to the solid phase. After rinsing the column the radioactivity of the column was measured. For calcns. a standard curve was established as given for the com. available TRAK-**Assay**.

L11 ANSWER 8 OF 14 MEDLINE on STN DUPLICATE 2
1998060950. PubMed ID: 9398703. Characterization of **monoclonal** thyroid-stimulating and thyrotropin binding-inhibiting autoantibodies from a Hashimoto's patient whose children had intrauterine and neonatal thyroid disease. Kohn L D; Suzuki K; Hoffman W H; Tombaccini D; Marcocci C; Shimojo N; Watanabe Y; Amino N; Cho B Y; Kohno Y; Hirai A; Tahara K. (Cell Regulation Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.. lenk@bdg10.NIDDK.nih.gov) . Journal of clinical endocrinology and metabolism, (1997 Dec) 82 (12) 3998-4009. Journal code: 0375362. ISSN:

0021-972X. Pub. country: United States. Language: English.

AB A multiplicity of **TSH receptor autoantibodies** (TSHRAbs) have been characterized after subcloning heterohybridomas produced from the lymphocytes of a patient who has Hashimoto's thyroiditis and had three children with intrauterine or neonatal hyperthyroidism. Twelve clones produced stimulating TSHRAbs that increased cAMP levels and iodide uptake in rat FRTL-5 thyroid cells and increased cAMP levels in Chinese hamster ovary (CHO) cells transfected with the human TSHR; like 95% of Graves' stimulating TSHRAbs, all 12 have their functional epitope on the N-terminus of the TSHR extracellular domain, requiring residues 90-165 for activity. All 12 bind to human thyroid membranes in the absence, but not the presence, of TSH, but are only weak inhibitors of TSH binding in **assays** measuring TSH binding-inhibiting Igs (TBIIs). In contrast, 8 different clones produced TSHRAbs that did not increase cAMP levels, but, instead, exhibited significant TBII activity. Four inhibited the ability of TSH or a stimulating TSHRAb to increase cAMP levels and had their functional epitope on the C-terminal portion of the TSHR external domain, residues 261-370, mimicking the properties of blocking TSHRAbs that cause hypothyroidism in patients with idiopathic myxedema. The 4 other TBIIs inhibited the ability of TSH, but not that of a stimulating TSHRAb, to increase cAMP levels, like TBIIs in Graves' patients. The functional epitope for 3 of these Graves'-like TBIIs was residues 90-165; the functional epitope for the fourth was residues 24-89. The fourth also increased arachidonic acid release and inositol phosphate levels in FRTL-5 thyroid cells and exhibited conversion activity, i.e. the ability to increase cAMP levels in the presence of an anti-human IgG. Thus, this TBII exhibited signal transduction activity, unlike the other 3 Graves'-like TBIIs. The patient, therefore, has stimulating TSHRAbs and 3 different types of TBIIs, each with different functional properties and different epitopes on the TSHR.

L11 ANSWER 9 OF 14 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

97:251175 The Genuine Article (R) Number: WP335. Folding-dependent binding of thyrotropin (TSH) and **TSH receptor autoantibodies** to the murine TSH receptor ectodomain. Vlase H; Matsuoka N; Graves P N; Magnusson R P; Davies T F (Reprint). CUNY, MT SINAI MED CTR, BOX 1055, 1 GUSTAVE L LEVY PL, NEW YORK, NY 10029 (Reprint); CUNY MT SINAI SCH MED, DEPT MED, NEW YORK, NY 10029; CUNY MT SINAI SCH MED, DEPT PHARMACOL, NEW YORK, NY 10029. ENDOCRINOLOGY (APR 1997) Vol. 138, No. 4, pp. 1658-1666. Publisher: ENDOCRINE SOC. 4350 EAST WEST HIGHWAY SUITE 500, BETHESDA, MD 20814-4110. ISSN: 0013-7227. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The mouse TSH receptor ectodomain (mTSHR-ecd) was amplified from murine thyroid complementary DNA and ligated into the pAcGP67B insect cell vector, and the nucleotide sequence was confirmed. Employing a baculovirus-insect cell system, the mTSHR-ecd (amino acids 22-415) was expressed as a fusion protein with the gp67 insect cell signal sequence at the NH2-terminus and a C-terminal six-histidine tag. Protein expression was assessed by Western blot using a murine **monoclonal** antibody (recognizing amino acids 22-35) and a rabbit antipeptide antibody (recognizing amino acids 397-415). These antibodies detected two principal species of mTSHR-ecd, one glycosylated (66 kDa) and one nonglycosylated (52 kDa), in cell lysates of infected insect cells. More than 10% of these species were present in a water-soluble (cytosolic) fraction. This fraction was then used to purify, under native conditions, 100-mu g amounts of mTSHR-ecd using nickel-nitrilo-triacetic (Ni-NTA) resin chromatography. The purified cytosolic mTSHR-ecd migrated as a homogeneous 66-KDa band visible on Coomassie blue-stained gels and was confirmed by Western blotting. We also purified the mTSHR-ecd from total cell lysates under denaturing conditions, followed by ''in vitro'' refolding on the Ni-NTA column. Under these conditions, milligram amounts of soluble mTSHR-ecd were obtained. This material consisted primarily of the 66-kDa glycosylated form, but in addition contained four or five lower molecular mass, partially glycosylated intermediates and the 52-kDa nonglycosylated

form. Deglycosylation with either endoglycosidase F or H, reduced all mTSHR-ecd glycosylated species to a 52-kDa nonglycosylated form. Both the cytosolic and refolded mTSHR-ecd preparations inhibited the binding of [I-125]TSH to the full-length human TSHR expressed in Chinese hamster ovary cells in a dose-dependent manner, with similar affinities. The affinity of such interactions was 3 orders of magnitude less than observed with native porcine TSHR and was further reduced by unfolding the mTSHR-ecd preparations. The cytosolic and refolded mTSHR-ecd were also recognized by hT-SHR autoantibodies in the serum of patients with hyperthyroid Graves' disease. Such autoantibody binding to mTSHR-ecd was also markedly reduced by unfolding the antigen.

These results demonstrated the successful production of large quantities of well characterized, biologically active, mTSHR-ecd antigen. In addition, the data showed that although the ectodomain of the mTSHR bound TSH, intact holoreceptor may be required for high affinity Ligand binding. Whether the transmembrane region is required for direct ligand binding, as seen for other G protein-linked receptors, or whether it is needed to stabilize the ligand binding to the ectodomain and maintain a correctly folded state, remains unclear.

L11 ANSWER 10 OF 14 MEDLINE on STN DUPLICATE 3

97255262. PubMed ID: 9100609. Thyrotropin (**TSH**) **receptor autoantibodies** do not appear to bind to the TSH receptor produced in an in vitro transcription/translation system. Prentice L; Sanders J F; Perez M; Kato R; Sawicka J; Oda Y; Jaskolski D; Furmaniak J; Smith B R. (FIRS Laboratories, RSR Ltd, Llanishen, Cardiff, United Kingdom.) Journal of clinical endocrinology and metabolism, (1997 Apr) 82 (4) 1288-92. Journal code: 0375362. ISSN: 0021-972X. Pub. country: United States. Language: English.

AB An in vitro transcription/translation (TnT) system was used to produce 35S-labeled full-length TSH receptor (TSHR) and TSHR extracellular domain (TSHRex). The interaction of the labeled proteins with TSHR autoantibodies in Graves' sera was then studied using an immunoprecipitation **assay**. In the **assay**, 35S-labeled TSHR or TSHRex were incubated with test sera, and any immune complexes formed were precipitated with protein A-Sepharose (in the case of mouse **monoclonal** antibodies, antimouse IgG-agarose was used). Rabbit antibodies to the TSHR and a mouse **monoclonal** antibody precipitated as much as 50% of the 35S-labeled TSHR preparations compared with about 2% for normal rabbit serum and 4% for a control **monoclonal** antibody. However, none of 34 Graves' sera (TSHR autoantibody levels ranging from 14-95% inhibition of [125I]TSH binding) were able specifically to immunoprecipitate 35S-labeled TSHR or TSHRex. These negative findings were confirmed by analysis of the immunoprecipitates on SDS-PAGE followed by autoradiography. Our results indicate that the TnT system is not useful for producing labeled TSHR preparations that can bind TSHR autoantibodies well. This is in contrast to TnT produced 35S-labeled glutamic acid decarboxylase, thyroid peroxidase, and 21-hydroxylase, which react well with their respective autoantibodies. One main difference between these 3 autoantigens and the TSHR is that the receptor is highly glycosylated, and this extensive glycosylation may be of critical importance for correct folding of the receptor. Consequently, the inability of the TnT system to glycosylate proteins could explain in part why TnT-produced 35S-labeled TSHR and TSHRex do not bind TSHR autoantibodies.

L11 ANSWER 11 OF 14 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

96:312751 The Genuine Article (R) Number: UF255. THE ANTIBODIES CAUSING THYROID-STIMULATING HORMONE-BINDING INHIBITION (TSH-BI) ARE NOT RESPONSIBLE FOR THE SPECIFIC-INHIBITION OF GONADAL STEROIDOGENESIS BY GRAVES SERA. CASTEL M A; WILLEY K P (Reprint); HUNT N; LEIDENBERGER F. UNIV HAMBURG, INST HORMONE & FERTIL RES, GRANDWEG 64, D-22529 HAMBURG, GERMANY (Reprint); UNIV HAMBURG, INST HORMONE & FERTIL RES, D-22529 HAMBURG, GERMANY. JOURNAL OF REPRODUCTIVE IMMUNOLOGY (FEB 1996) Vol. 30, No. 1, pp. 1-15. ISSN: 0165-0378. Pub. country: GERMANY. Language: ENGLISH

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Graves' disease is attributed to the presence of autoantibodies with agonist activity which interact with the TSH receptor causing thyroid hyperstimulation and hyperthyroidism. The degree of TSH-binding inhibition (TSH-BI) caused by a Graves' serum in a TSH radioligand receptor **assay** is considered to be an index of the prevalence of anti-**TSH receptor autoantibodies** in that serum. We have previously shown that the specific inhibition by Graves' serum of hCG-stimulated steroidogenesis by Leydig cells was at a site distal to receptor binding and second messenger activation. In this report, we have investigated whether the effect of Graves' serum upon Leydig cells is a property of the constitutive antibodies. Immunoglobulin-enriched fractions were obtained from Graves' and normal sera using three increasingly rigorous procedures; ammonium sulphate precipitation, caprylic acid treatment and Protein A or G-affinity purification. The TSH-BI was determined for untreated and extracted sera in two radioreceptor **assays** developed for use with serum, one using human thyroid membranes and the other using HeLa cells transfected with the human TSH receptor, and the results were compared with effects in the Leydig cell steroidogenesis bioassay. The specific inhibition of hCG-stimulated Leydig cell steroidogenesis by Graves' sera was not retained in the antibody fraction causing TSH-BI. Thus, the inhibitory factor appears not to be an antibody and we are now attempting to purify and identify the responsible factor from Graves' serum.

L11 ANSWER 12 OF 14 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN

92:168899 The Genuine Article (R) Number: HH583. IDENTIFICATION OF SEPARATE DETERMINANTS ON THE THYROTROPIN RECEPTOR REACTIVE WITH GRAVES THYROID-STIMULATING ANTIBODIES AND WITH THYROID-STIMULATING BLOCKING ANTIBODIES IN IDIOPATHIC MYXEDEMA - THESE DETERMINANTS HAVE NO HOMOLOGOUS SEQUENCE ON GONADOTROPIN RECEPTORS. KOSUGI S; BAN T; AKAMIZU T; KOHN L D (Reprint). NIDDKD, BIOCHEM & METAB LAB, CELL REGULAT SECT, BETHESDA, MD, 20892. MOLECULAR ENDOCRINOLOGY (FEB 1992) Vol. 6, No. 2, pp. 168-180. ISSN: 0888-8809. Pub. country: USA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Deletions, substitutions, or mutations of the rat TSH receptor extracellular domain between residues 20 and 107 (all residue numbers are determined by counting from the methionine start site) have been made by site-directed mutagenesis of receptor cDNA. After transfection in Cos-7 cells, constructs were evaluated for their ability to bind [I-125]TSH or respond to TSH and thyroid-stimulating antibodies (TSAbs) from Graves' patients in **assays** measuring cAMP levels of the transfected cells. **Assay** results were compared to results from Cos-7 cells transfected with wild-type receptor constructs or vector alone. We identify threonine-40 as a TSAb-specific site whose mutation to asparagine, but not alanine, reduces TSAb activity 10-fold, but only minimally affects TSH-increased cAMP levels. We show that thyroid-stimulating blocking antibodies (TSBAbs), which block TSH or TSAb activity and are found in hypothyroid patients with idiopathic myxedema, continue to inhibit TSH-stimulated cAMP levels when threonine-40 is mutated to asparagine or alanine, suggesting that TSBAbs interact with different TSH receptor epitopes than the TSAb autoantibodies in Graves' patients. This is confirmed by the demonstration that these TSBAbs interact with high affinity TSH-binding sites previously identified at tyrosine-385 or at residues 295-306 of the extracellular domain of the TSH receptor. This is evidenced by a loss in the ability of TSBAbs to inhibit TSAb activity when these residues are mutated or deleted, respectively. Since the TSAb and TSBAb epitopes are in regions of the extracellular domain of the TSH receptor that have no homology in gonadotropin receptors, these data explain at least in part the organ-specific nature of **TSH receptor autoantibodies** in autoimmune thyroid disease. Data are additionally provided which indicate that residues 30-37 and 42-45, which flank the TSAb epitope at threonine-40, appear to be ligand interaction sites more important for high affinity TSH

binding than for the ability of TSH to increase cAMP levels and that cysteine-41 is critical for TSH receptor conformation and expression on the surface of the cell. Thus, despite unchanged maximal values for TSH-increased cAMP levels, substitution of residues 42-45 or deletion of residues 30-37 results in receptors, which, by comparison to wild-type constructs, exhibit significantly worsened K(d) values for TSH binding than EC50 values for TSH- or TSAb-increased cAMP activity. Mutation of cysteine-41 to serine, however, results in a receptor that expresses no TSH binding or TSH- or TSAb-increased cAMP activity; its deletion, with residue 42, produces a receptor that retains some TSH- or TSAb-increased cAMP activity, but exhibits reduced maximal activity. Mutation of cysteine-31 to serine, in contrast, increases TSH binding as well as both TSH- and TSAb-increased cAMP levels. Residues flanking threonine-40, thus appear to be important for ligand interaction as well as receptor conformation.

L11 ANSWER 13 OF 14 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
91:375377 The Genuine Article (R) Number: FU443. BINDING DOMAINS OF STIMULATORY AND INHIBITORY THYROTROPIN (**TSH**) **RECEPTOR AUTOANTIBODIES** DETERMINED WITH CHIMERIC TSH-LUTROPIN CHORIONIC-GONADOTROPIN RECEPTORS. NAGAYAMA Y (Reprint); WADSWORTH H L; RUSSO D; CHAZENBALK G D; RAPOPORT B. VET ADM MED CTR, THYROID MOLEC BIOL UNIT 111T, 4150 CLEMONT ST, SAN FRANCISCO, CA, 94121 (Reprint); UNIV CALIF SAN FRANCISCO, SAN FRANCISCO, CA, 94143. JOURNAL OF CLINICAL INVESTIGATION (1991) Vol. 88, No. 1, pp. 336-340. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB We examined the relative effects of thyrotropin (TSH) and **TSH receptor autoantibodies** in the sera of patients with autoimmune thyroid disease on three TSH-lutropin/chorionic gonadotropin (LH/CG) receptor extracellular domain chimeras. Each chimera binds TSH with high affinity. Only the chimera with TSH receptor extracellular domains ABC (amino acids 1-260) had a functional (cAMP) response to thyroid stimulatory IgG. The chimeras with TSH receptor domains CD (amino acids 171-360) and DE (amino acids 261-418) were unresponsive. The lack of response of the chimera with TSH receptor domains DE was anticipated because it fails to transduce a signal with TSH stimulation, unlike the other two chimeras. A different spectrum of responses occurred when the TSH-LH/CG chimeras were examined in terms of autoantibody competition for TSH binding. IgG with TSH binding-inhibitory activity when tested with the wild-type TSH receptor also inhibited TSH binding to the chimera with TSH receptor domains DE. Dramatically, however, these IgG did not inhibit TSH binding to the chimera with TSH receptor domains CD, and had weak or absent activity with the chimera with TSH receptor domains ABC. Chimeras with TSH receptor domains ABC and DE were equally effective in affinity-purifying IgG with thyroid-stimulatory and TSH binding-inhibitory activities. Nonstimulatory IgG with TSH binding-inhibitory activity inhibited the action of stimulatory IgG on the wild-type TSH receptor, but not with the chimera containing TSH receptor domains ABC. In summary, **TSH receptor autoantibodies** and TSH bind to regions in both domains ABC and DE of the TSH receptor extracellular region. Stimulatory and inhibitory **TSH receptor autoantibodies**, as well as TSH, appear to bind to different sites in domains ABC, but similar sites in domains DE, of the receptor. Alternatively, TSH and the different TSH receptor antibodies bind with differing affinities to the same site in the ABC region.

L11 ANSWER 14 OF 14 MEDLINE on STN DUPLICATE 4
87063678. PubMed ID: 2878349. Further evaluation of an immunoprecipitation **assay** for **TSH-receptor autoantibodies** in Graves' disease. De Bruin T W; Braverman L E; Brown R S. Metabolism: clinical and experimental, (1986 Dec) 35 (12) 1101-5. Journal code: 0375267. ISSN: 0026-0495. Pub. country: United States. Language: English.
AB In ten patients with untreated Graves' disease, quantitative titers of TSH receptor antibodies, as measured by a recently developed immunoprecipitation **assay** (IPA), were correlated with results

obtained in three other methods and with the severity of the hyperthyroidism, as assessed by thyroid function tests. Nine patients were positive in the IPA, six in the TBII (TSH-binding inhibitor immunoglobulins), six in the TSI (thyroid-stimulating immunoglobulins), and seven in the TGI (thyroid-growth stimulating immunoglobulins) **assay.** Three patients were positive in all four **assays.** No correlation was found between the IPA values and the results obtained in the TBII, TSI, or TGI **assays.** There was a modest correlation between the TBII and TSI **assays** (r = .74, P less than 0.02). There was a modest but significant correlation between the IPA titers of TSH receptor antibodies and serum T3 concentration, both before treatment (r = .63, P less than 0.05) and during treatment (n = 5; r = .84, P less than 0.05). No correlation between the severity of the hyperthyroidism and TBII, TSI, or TGI **assays** was observed. Finally, TGI did not correlate with goiter size as estimated by palpation. These results suggest that the IPA may be useful in monitoring the immunologic activity of TSH receptor antibodies in patients with Graves' disease. They suggest further that the IPA detects a number of different subpopulations of TSH receptor antibodies, including TSI, TBII, and perhaps TGI. This property may be particularly useful in screening for **monoclonal** TSH receptor antibodies.

```
=> s l8 and bovine TSH
L12          15 L8 AND BOVINE TSH

=> dup remove l12
PROCESSING COMPLETED FOR L12
L13           4 DUP REMOVE L12 (11 DUPLICATES REMOVED)

=> d l13 1-4 cbib abs
```

L13 ANSWER 1 OF 4 MEDLINE on STN DUPLICATE 1

2003575686. PubMed ID: 14654252. Affinity purification and diagnostic use of **TSH receptor autoantibodies** from human serum. Morgenthaler Nils G; Minich Waldemar B; Willnich Marita; Bogusch Thomas; Hollidt Jorg M; Weglohner Wolfgang; Lenzner Cornelia; Bergmann Andreas. (Research Department of BRAHMS AG, Biotechnology Center Hennigsdorf/Berlin, Neuendorfstr. 25, D-16761 Hennigsdorf, Germany.. n.morgenthaler@brahms.de) . Molecular and cellular endocrinology, (2003 Dec 30) 212 (1-2) 73-9. Journal code: 7500844. ISSN: 0303-7207. Pub. country: Ireland. Language: English.

AB Purification of **TSH receptor autoantibodies** (TRAb) from the serum of patients with Graves' disease (GD) might help to elucidate the nature of these disease causing autoantibodies. We describe here for the first time the successful affinity purification of human TRAb.Affinity purification was performed in a four step procedure with human recombinant TSH receptor (TSH-R) expressed in K562 cells. Purification from six different serum pools from patients with GD and two individual sera (one with only thyroid stimulating antibodies (TSAb) one with only thyroid blocking antibodies (TBAb)) resulted in a purity of 39.2+/-3.8 IU/mg TRAb or 25.7+/-2.1 microg IgG/IU (about 3.5-13.7 microg TRAb/ml serum). The average enrichment based on the respective original serum was 3420-fold (range 1200-10,000). The kDa of the purified TRAb were in the range of 0.7-2.6 x 10(-10)M. All purified TRAb (except from the TBAb serum which showed blocking activity) showed a more than 1000-fold stronger stimulation in the TSAb bioassay based on the IgG content than the original serum, and similar stimulation based on international units (IU/l) TRAb. When labelled purified TRAb were used in a competitive **assay** as tracer instead of **bovine TSH**, their binding to the human recombinant TSH-R on tubes was displaced by 99 of 100 GD sera (selected for TBII activity). Correlation to the standard TSH tracer was r=0.92. Interestingly, the use of TRAb tracer derived from a patient with TSAb and a patient with TBAb gave virtually identical results (r=0.93) with these patients, suggesting

similar if not identical binding sites for both TRAb subtypes.In conclusion, this is the first report on the purification of human TRAb from the serum of patients with GD. The purified TRAb are of low concentration with high affinity, strong TBII and TSAb activity. Further characterisation may allow new insights in TRAb epitope localisation, the pathology of GD and the differences between TSAb and TBAb. Also, their use as tracer in a competitive **assay** is the first report on a completely homogenous **assay** with high sensitivity for TSH-R autoantibodies.

L13 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN
2000:14177 Document No. 132:292289 New **assay** systems for thyrotropin receptor antibodies. Morgenthaler, Nils G. (Research Department, B.R.A.H.M.S. Diagnostica GmbH, Berlin, 12099, Germany). Current Opinion in Endocrinology & Diabetes, 6(4), 251-260 (English) 1999. CODEN: CENDES. ISSN: 1068-3097. Publisher: Lippincott Williams & Wilkins.

AB A review with 74 refs. The detection of autoantibodies to the TSH receptor is a useful tool for the diagnosis of Graves' disease. Historically, there are two established methods for this purpose. One is the radioreceptor **assay** based on the porcine TSH-R, where autoantibodies and labeled **bovine TSH** compete for the binding sites of the receptor. The other method is based on the ability of some autoantibodies similar to TSH to induce the second messenger cAMP in certain cell lines. These so-called bioassays are able to distinguish between stimulating or blocking autoantibodies, based on their biol. activity, to either enhance or inhibit the cAMP production Ten years after the cloning of the human TSH-R, these two basic detection principles were finally improved. In this article, the author summarizes the latest developments in TSH-R autoantibody **assay** technol. and outlines the current research on alternative approaches, such as direct-binding **assays.** Controversies related to autoantibody terminol. and **assay** interpretation are also addressed.

L13 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 2
97358163. PubMed ID: 9215283. The human thyrotropin (TSH) receptor in a TSH binding inhibition **assay** for **TSH receptor autoantibodies.** Kakinuma A; Chazenbalk G D; Jaume J C; Rapoport B; McLachlan S M. (Thyroid Molecular Biology Unit, Veterans Administration Medical Center, San Francisco, California 94121, USA.) Journal of clinical endocrinology and metabolism, (1997 Jul) 82 (7) 2129-34. Journal code: 0375362. ISSN: 0021-972X. Pub. country: United States. Language: English.

AB Seven years after the molecular cloning of the human TSH receptor (TSHR), the porcine TSHR remains in general use in the TSH binding inhibition (TBI) **assay** for autoantibodies to the TSHR. We compared porcine and recombinant human TSHR in two types of TBI **assays**: one using intact Chinese hamster ovary cells expressing the recombinant human TSHR on their surface, and the other using soluble receptors extracted from these cells with detergent. In the intact cell TBI **assay**, monolayers expressing large numbers of TSHR were less effective than cells expressing few receptors. These findings are consistent with the very low concentration of TSHR autoantibodies in serum. Binding of [125I]human TSH was about 5-fold lower than that of [125I]**bovine TSH** to the intact cells. Nevertheless, TBI values with the two ligands were similar for most sera. However, a few sera produced greater inhibition of human than of **bovine TSH** binding. In the solubilized human TSHR TBI **assay**, in contrast to the intact cell TBI **assay**, cells expressing very large number of TSHR were an excellent source for detergent extraction of soluble human TSHR, but only if the cells were extracted while still on the dish and not after scraping. A 10-cm diameter dish of cells provided TSHR for 100-200 replicate determinations when substituted for solubilized porcine TSHR in a commercial TBI kit. TBI values in serum from 30 individuals with suspected Graves' disease correlated closely when tested with solubilized

human and porcine TSHR (r = 0.954; P < 0.001). However, 2 sera that were negative with the porcine TSHR were positive with the human TSHR. TBI and thyroid-stimulating activity in these sera correlated weakly regardless of whether the TBI used human or porcine TSHR. These findings open the way to a practical TBI **assay** using recombinant human TSHR.

L13 ANSWER 4 OF 4 MEDLINE on STN DUPLICATE 3
88190770. PubMed ID: 3358096. Evaluation of a radioreceptor **assay** for **TSH receptor autoantibodies.** Rootwelt K. (Institute of Clinical Biochemistry, Rikshopitalet, University of Oslo, Norway.) Scandinavian journal of clinical and laboratory investigation, (1988 Apr) 48 (2) 157-64. Journal code: 0404375. ISSN: 0036-5513. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A commercial radioreceptor **assay** for **TSH receptor autoantibodies** (TRAb), based on solubilized porcine receptor and purified radio-iodinated **bovine TSH**, was tested in 264 subjects with a variety of thyroid disorders. The sensitivity of the **assay** for the detection of hyperthyroid Graves' disease was 91%. The **assay** specificity for Graves' disease was 95%. With the exception of one patient with Hashimoto's disease and one patient with de Quervain's subacute thyroiditis no subjects other than Graves' patients had detectable TRAb. Thus purely blocking **TSH receptor autoantibodies** were not detected with the **assay.** One female with thyroxine-treated idiopathic primary hypothyroidism who had given birth to two children with transiently elevated TSH, was found to have a circulating TSH-binding substance that resulted in an abnormally negative TRAb value, and highly discrepant results when TSH was measured with a double antibody TSH radioimmunoassay and an immunoradiometric **assay.** The TSH-binding substance was precipitated like a protein, but was not IgG. Similar findings have not previously been reported.

=> s (bergmann a?/au or struck j?/au or morgenthaler n?/au or weglohner w?/au or hollidt j?/au)
L14 1209 (BERGMANN A?/AU OR STRUCK J?/AU OR MORGENTHALER N?/AU OR WEGLOHNER W?/AU OR HOLLIDT J?/AU)

=> s l14 and TSH receptor assay
L15 1 L14 AND TSH RECEPTOR ASSAY

=> d l15 cbib abs

L15 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN
1994:427925 Document No. 121:27925 Purified TSH prepn., methods of its production and its use for the production of TSH tracers for **TSH receptor assays** and in **TSH receptor assays. Bergmann, Andreas; Struck, Joachim** (Henning Berlin GmbH Chemie- und Pharmawerk, Germany). Ger. DE 4237430 C1 19940317, 10 pp. (German). CODEN: GWXXAW. APPLICATION: DE 1992-4237430 19921105.

AB A com. crude bovine TSH preparation was purified by affinity chromatog. on an anti-TSH antibody affinity column followed by ion exchange chromatog. on a polyamide-coated silica gel column. The biol. activity of the purified preparation was >40 IU TSH/mg protein. The purified TSH preparation was radioiodinated to produce a tracer which was used especially in TSH receptor autoantibody assays.

=> s l14 and TSH monoclonal
L16 1 L14 AND TSH MONOCLONAL

=> d l16 cbib abs

L16 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

1996:301493 Document No. 124:333331 Monoclonal antibodies to the human TSH receptor: epitope mapping and binding to the native receptor on the basolateral plasma membrane of thyroid follicular cells. Nicholson, L. B.; Vlase, H.; Graves, P.; Nilsson, M.; Molne, J.; Huang, G. C.; **Morgenthaler, N. G.**; Davies, T. F.; McGregor, A. M.; Banga, J. P. (Dep. Med., King's College Sch. Med., London, SE5 9PJ, UK). Journal of Molecular Endocrinology, 16(2), 159-170 (English) 1996. CODEN: JMLEEI. ISSN: 0952-5041. Publisher: Journal of Endocrinology.

AB We have characterized four murine monoclonal antibodies (mAbs) to the extracellular domain of the human TSH receptor (TSH-R.E), the target autoantigen of Graves' disease. Recombinant TSH-R.E used as immunogen, was produced in E. coli as fusion protein with glutathione-S-transferase of in a baculovirus-insect cell system, as a non-fusion glycoprotein. To increase the epitope specificity of the mAbs, two different strains of mice (H-2b and H-2d) were immunized. The epitopes recognized by the mAbs were characterized by immunoblotting with various recombinant constructs of TSH-R.E and by binding to overlapping synthetic peptides of the receptor. The four IgG mAbs characterized recognized epitopes localized to different regions on the TSH-R.E; amino acids 22-35 (A10 and A11, both IgG2b from H-2b animals), amino acids 402-415 (A7, IgG2b from H-2b animals) and amino acids 147-228 (A9, IgG1 from H-2d animals). Immunolocalization studies showed that mAb A9 recognized TSH-R.E on unfixed cryostat sections, where binding was localized to the basolateral plasma membrane of thyroid follicular cells, suggesting that this antibody reacts with the native receptor on thyroid cells. The binding of the mAbs A7, A10 and A11 was also restricted to the basal surface of thyroid cells, but only after acetone fixation of the sections, implying that the epitopes recognized on the amino and carboxyl terminus of the extracellular region of the receptor are not accessible on the native mol. None of the mAbs stimulated cAMP responses in COS-7 cells transiently transfected with full-length functioning TSH-R.E, while weak inhibition of binding of radiolabeled TSH to porcine membranes in a radioreceptor assay was apparent with mAb A10 and A11, but only at high concns. of IgG. The ability of mAb A9 to bind to the native receptor without stimulating activity or inhibition of TSH binding suggests that antibody can bind to the central region of the TSH-R.E without perturbing receptor function. The availability of mAbs that recognize epitopes on different regions of the extracellular domain of TSH-R will lead to a better understanding of the autoantigenic regions on TSH-R implicated in disease activity.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	135.39	135.60

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-5.54	-5.54

STN INTERNATIONAL LOGOFF AT 13:01:39 ON 16 JUN 2004